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I would like to request the following reference:

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Relationship of blood clotting and tumor angiogenesis.
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Thank you.

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Haemostasis

Lectures

Haemostasis 2001;31(suppl. 1):16-20

Angiogenesis

Relationship of blood clotting and tumor angiogenesis

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Tissue factor (TF), the 47 KDa transmembrane protein activator of blood coagulation, has additional biologic functions, including the promotion of tumor angiogenesis [1-3] and cell adhesion [4]. The bifunctional properties of TF as both a pro-clotting and an adhesive and pro-angiogenic protein probably contributes to its important role in the enhancement of tumor growth and metastasis [3,5]. This relationship of blood clotting to tumor angiogenesis extends further in the hemostatic system to the platelet release reaction, soluble clotting factors and their inhibitors, cross-linked fibrin and, finally to the fibrinolytic cascade, a rich source of both positive and negative regulators of angiogenesis [6]. Perhaps, given the important role of platelet, clotting and fibrinolytic proteins in the maintenance of the hemostatic plug, and tissue and vascular repair processes, it should not surprise us that one or more of these proteins can also serve to either enhance or inhibit new vessel formation [Figure; Reference 7].

As has been reviewed in preceding papers, tumor cells themselves possess a variety of procoagulant properties, including the constitutive, cell surface expression of TF, the secretion of the cysteine protease, cancer procoagulant (CP), that directly cleaves factor X, and procoagulant cytokines such as IL-1, IL-8 and vascular endothelial growth factor (VEGF). Tumor cell VEGF is chemotactic for both macrophages and endothelial cells and activates TF in both types of cell. Tumor cells also activate platelets and, via integrin expression, form adhesive interactions with platelets and the endothelium of blood vessels. Tumor cell interaction with the vessel wall reduces endothelial cell secretion of tissue plasminogen activator (TPA) and expression of thrombomodulin (TM), and increases endothelial cell synthesis of plasminogen activator inhibitor (PAI-1). Finally, substantial experimental evidence supports the presence of increased numbers of activated monocytes/macrophages in the circulation of cancer patients and in proximity to growing tumors. These antigen-processing cells express TF on their surface, presumably as part of the host immune response to the tumor and/or in response to secretion of tumor products. Tumor-associated macrophages have been shown to assemble the entire coagulation cascade and form cross-linked fibrin on their surface in apposition to growing tumor. The activation of coagulation in the tumor microenvironment, which routinely spills into the circulation of cancer patients, rendering them "hypercoagulable", may be a primitive effort on the part of the host to limit the spread of tumor cells [3]. Macrophage TF expression in cancer patients (measured in cultured peripheral blood monocytes) correlates significantly with plasma levels of the thrombin-induced, fibrinogen activation peptide, fibrinopeptide A (FPA). Although correlation never proves causation, it is notable that cross-linked fibrin (XLF) can be co-localized with TF in both tumor-associated macrophages and within the endothelium of tumor-associated blood vessels in human breast and lung cancer. The finding of TF expression in vascular endothelial cells (VECs) in proximity to or within a growing tumor lends further support for the concept that new vessels formed as a result of angiogenic signals generated by tumors may be more

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susceptible to thrombogenesis. Further, this observation stimulated further exploration of a possible role for TF in the development of neoangiogenesis. Interleukin-8 is a potent pro-angiogenic cytokine elaborated by a variety of cells, including VECs. Cross-linked fibrin formed on the surface of VECs up-regulates the gene for IL-8 and induces the synthesis and release of increasing concentrations of functional IL-8 from VECs in a dose-dependent fashion. XLF also induces the expression of the TF gene in VECs at concentrations readily achievable in vivo and in the absence of thrombin. Therefore, it seems likely that multiple products of blood coagulation, including both thrombin and fibrin, can stimulate the activation and migration of endothelial cells (perhaps via IL-8 release) and induce a self-perpetuating system for further activation of blood coagulation (by induction of TF). Co-localization of TF and VEGF genes and functional antigens in situ in human lung and breast cancer cells further supports the potential for joint regulation of both clotting reactions and angiogenesis in human tumors [7,8].

A unifying hypothesis that could link the expression of TF and the development of new blood vessel formation in tumors was suggested by experiments in murine tumors described by Zhang and colleagues [1]. These investigators demonstrated that expression of VEGF in murine tumor cells was upregulated by transfection of the gene for TF into the cells. Furthermore, the level of the anti-angiogenic peptide thrombospondin varied inversely in the cells with the expression of TF. Once transplanted into a syngeneic murine recipient, tumors expressing high levels of the TF gene became highly vascularized. Neo-angiogenesis occurred in spite of maximal anticoagulation of the recipient mice or pretreatment of the tumor cells in vitro to minimize the potential for coagulation activation following transplantation. These results clearly suggested a new role for TF in the stimulation of angiogenesis, independent of its role as the primary trigger of blood coagulation. We extended these experiments to human breast and melanoma tumors grown as xenografts in immunodeficient mice. In 10 human breast cancer cell lines and 13 human melanoma cell lines, we demonstrated a strong correlation between levels of TF and VEGF generated in vitro ($r = 0.84$, $p < 0.0001$; $r = 0.94$, $p < 0.0001$, respectively) [2]. We selected, respectively, a high TF and VEGF producer (RPMI-7951) and a low producer (WM-115) melanoma cell line for further study.

Both the high producer and low producer cell lines generated tumors in SCID mice. The high producer cell lines generally resulted in highly vascular tumors within 8-10 weeks, as determined by immunohistochemical analysis with either anti-von Willebrand factor or anti-CD31 antibodies. Inoculation of SCID mice with the low producer cell lines resulted in rather avascular tumors on gross inspection with scant numbers of visible blood vessels by immunohistochemical analysis of at least 10, 200x microscopic fields by a blinded observer (RPMI-7951 = 17.3 ± 4.0 vs. WM-115 = 3.2 ± 2.2; $p = 0.01$) [2]. To test the hypothesis that this observed difference in vascularity in vivo between high TF-expressing and low TF-expressing tumors might be the result of molecular regulation of VEGF expression by TF, we transfected full-length TF cDNA in the sense orientation into another low producer melanoma cell line, HT-144 and analyzed the cells in vitro for both TF and VEGF production. Successful transfection of the full-length TF cDNA into the HT144 endogenous low producer cell line restored synthesis of both TF and VEGF to normal levels, supporting the hypothesis that TF somehow participates in the regulation of VEGF synthesis [2]. Consistent with data published by Bromberg and colleagues in a mouse tumor model after intravenous injection of melanoma cells [9], a TF mutant lacking the cytoplasmic domain (but retaining full procoagulant function) failed to sustain VEGF production in vitro or angiogenesis in vivo in our model [2]. In control experiments, VEGF transcription was fully restored in the cells with either the full-length TF sequence or with an extracellular domain mutant sequence ($Y^{157} \rightarrow A^{157}$ and $K^{159} \rightarrow A^{159}$), the latter of which prevents successful TF-mediated factor X activation by the cells [2]. These data indicate that the TF cytoplasmic tail is sufficient to induce VEGF transcription and that factor VIIa/factor X interactions are not required in this model. The in vitro observations were confirmed in vivo in xenogeneic tumors in nude mice, in which tumors resulting from the wild type cells were generally avascular and those resulting from the cells containing the full-length TF transfectant were replete with CD31+ blood vessels. Having demonstrated that TF can regulate VEGF production in vitro and in vivo, we began to develop a strategy for delivering inhibitors of both TF and VEGF production to tumor cells that might result ultimately in a novel therapeutic approach to cancer therapy.

Other groups have successfully targeted TF in both tumor cells and VECs in experimental approaches to model tumors [10-12]. Zhang and colleagues [10] and Huang et al. [11] developed novel strategies for targeting the tumor vasculature, taking some

advantage of the apparently selective expression of TF in the neoangiogenic vessels of experimental murine tumors. Zhang et al. demonstrated that even in those tumors where the cancer cells themselves expressed little or no TF, the VECs expressed TF upon induction with tumor necrosis factor (TNF). TNF induction stimulated the production of fibrin in the vessels perfusing the tumor resulting in tumor necrosis. Blood flow was restored to the tumors with somatic gene transfer of anti-sense to TF. Huang et al. treated neuroblastomas in mice by intravenous adminis-tration of a bifunctional immunoconjugate consisting of a truncated, soluble form of TF (that retains only limited PCA) targeted with a monoclonal antibody to an expressed antigen specific for murine tumor endothelium [11]. This conjugate resulted in thrombosis of tumor vessels and complete tumor regression in 38% of the treated mice. A similar strategy as that employed by Huang et al. was employed successfully by Hu et al. [12], who regressed human melanoma in immunodeficient mice using an active site mutated factor VIIa conjugate that targeted TF in the endothelium of angiogenic vessels. We demonstrated previously that inactivated recombinant factor VIIa (rVIIai) is a specific and high affinity ligand for TF expressed on the surface of human tumor cells both in vitro and in situ. Factor VIIa mutated at the active site or inactivated with a tripeptide, chloromethyl ketone inhibitor retains its affinity for TF but is incapable of activating clotting. Factor VIIa binding to TF on cell surfaces also induces translocation of TF-VIIa complexes into caveolae, a phenomenon that is mediated equally well by rVIIai. We used rVIIai as a "Trojan Horse" to carry potential toxins into TF-bearing tumor cells and tumor-associated VECs. Curcumin [1,7-Bis(4-dydroxy-3methoxyphenyl)-1,6-heptadiene-3,5-dione], a major chemical component of the curry spice turmeric, has been found to have a variety of interesting biologic properties in various experimental systems. In particular curcumin blocks phorbol ester- and TNFα-induced TF synthesis by VECs by binding and inhibiting the action of the transcription factors NF-kB, AP-1 and Egr-1 [13,14] and curcumin inhibits angiogenesis in vivo [15]. In preliminary studies utilizing tumor cells and human VECs, we demonstrated that a series of synthetic curcumin analogs inhibit both TF and VEGF gene expression in vitro [16]. Studies are in progress to determine if one or more of the analogs linked to rVIIai can be utilized effectively to target TF and VEGF in tumor cells and tumor-associated blood vessels in vivo. It is our hope that this approach, which targets TF, might provide a bifunctional therapy for both the hypercoagulability and the excessive angiogenesis in cancer.